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TECHNICAL REPORT
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**LESSONS LEARNED ON BIOAUGMENTATION
OF DNAPL SOURCE ZONE AREAS**

ESTCP PROJECT: ER-0008

By
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14. ABSTRACT The ESTCP funded a pilot scale demonstration in which 100L of PCE were released in a test cell at Dover AFB. The purpose of the demonstration was to investigate the effects of biological activity on enhancing dissolution of the emplaced PCE source. Both laboratory and field tests were performed using biostimulation and bioaugmentation. Both laboratory and field efforts demonstrated that bioaugmentation can stimulate complete dechlorination to nontoxic end products. Lessons learned from the demonstration can be applied at other sites and include: a) the rationale for bioaugmentation, b) how to assess the need for bioaugmentation, and c) tracking system performance.					
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1. INTRODUCTION

This document has been prepared as part of the Environmental Security Technology Certification Program (ESTCP) Project ER-0008; Bioremediation of DNAPL Source Zone Areas through Bioaugmentation. The purpose is to guide Remedial Project Managers (RPMs) on the appropriateness, application and evaluation metrics of bioaugmentation to treat source areas of chlorinated volatile organic compounds (chlorinated VOCs) in groundwater, such as tetrachloroethene (PCE) and trichlorethene (TCE). This document would be appropriate for use at the Feasibility Study stage of a site remedial program. The document does not compare biostimulation alone against bioaugmentation, as the focus of ESTCP ER-0008 was to demonstrate that bioaugmentation within a PCE source was able to increase the dissolution rate of PCE free-phases to decrease source longevity and/or the mass flux of volatile organic compounds (VOCs) that form the dissolved phase plume. Furthermore, there are various documents available that review how to select biostimulation or bioaugmentation to treat dissolved phases of cVOCs in groundwater (www.itrcweb.org, AFCEE 2004).

1.1 Background

Technologies that focus on the removal and/or management of groundwater source areas containing dense, non-aqueous liquids (DNAPLs) of cVOCs are of increasing interest to the Department of Defense (DoD) as they minimize the long-term liability associated with groundwater contamination by reducing the source's longevity or mass loading into the dissolved phase plume and decrease the plume size and downgradient concentration of cVOCs over time.

Although, the relationship between the mass of DNAPL removed and the reduction in plume mass loading is not completely understood nor it is predictable, the value of this conceptual framework is that it suggests that there is a reduction in plume loading that is independent of the technology used for DNAPL removal. Accordingly, as source concentrations decrease, there is an opportunity to use lower cost, less aggressive treatment technologies that are better suited to the change in the source area concentrations caused by DNAPL mass removal.

As a result, numerous DNAPL remediation technologies have been developed to enhance more rapid DNAPL mass removal, typically through either enhanced solubilization or DNAPL mobilization. Enhanced solubilization is frequently achieved either by increasing mass transfer from the DNAPL via mass destruction (i.e. bioremediation, in situ chemical oxidation or thermal technologies) or via enhanced solubility (i.e. alcohol flushing, co-solvent flushing, or increased temperatures).

The rate at which a DNAPL dissolves is controlled by the mass transfer rate of the DNAPL from its non-aqueous to aqueous phase. The mass transfer rate is driven by the concentration gradient that exists at the DNAPL:water interface. The greatest mass transfer rate occurs where

the highest concentration gradient exists. Under natural systems, the maximum concentration gradient and dissolution rate occurs where clean groundwater first contacts the DNAPL. The leading edge of the DNAPL will dissolve to its maximum solubility limit and be transported downgradient where it will come into contact with DNAPLs that are already in local equilibrium with dissolved phases (i.e., DNAPLs have dissolved to their maximum solubility at the DNAPL:water interface). More DNAPL mass cannot be dissolved into solution because the downgradient DNAPLs are already in local equilibrium. Therefore, the DNAPL dissolves only at the leading edge, and why the overall DNAPL pool length has a strong effect on longevity on DNAPL sources. Many sites do not have continuous pools of DNAPL but rather have stringers, ganglia or blobs that can create an “effective pool length”. As the leading edge of these discontinuous DNAPL free-phases dissolves, higher dissolved phase concentrations will form over the remaining down gradient DNAPLs, slowing or preventing their dissolution.

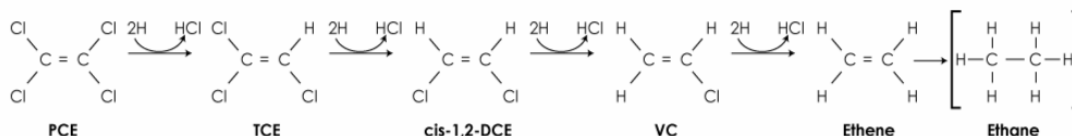
The concentration gradient and the resulting dissolution rate are a function of the DNAPL solubility, diffusion and partitioning coefficients, dispersion (mixing), and the effective pool length to ganglia ratio. Higher concentration gradients and resulting higher mass transfer rates of DNAPLs will occur in fast flowing groundwater systems that contain DNAPLs with high aqueous solubility, high surface area (low pool to ganglia ratio), and little sorbed mass. At the opposite extreme, low rates of mass transfer will occur when DNAPLs have low solubility, and low surface area, and where hydrogeological conditions make mass transfer diffusion controlled.

1.1.1 Biologically Based Enhanced Dissolution

The goal of bioremediation is to decrease the dissolved phase concentration of the VOC near: (i) the free-phase DNAPL:water interface; and/or (ii) in between DNAPL phases to create shorter effective pool lengths. As dechlorinating bacteria can survive, if not thrive, in the presence of DNAPL, their destruction of the dissolved chemicals dramatically increases the concentration gradient at the DNAPL:water interface and also in between the DNAPL free- and sorbed phases, which in turn allows for more rapid dissolution of the DNAPL within the source area. Even if enhanced dissolution cannot be achieved biologically near the DNAPL:water interface, rapid biodegradation of the high VOC concentrations typically encountered in DNAPL source zones (i.e. tens to hundreds of milligrams per liter [mg/L]) will provide biological containment of the source area.

A number of microorganisms have now been identified that are capable of utilizing either PCE or TCE as electron acceptors (dehalorespiration). To date, only members of the genus *Dehalococcoides* (*Dhc*) are known to be capable of metabolic utilization of cis-1,2-dichloroethene (cis-1,2-DCE) or vinyl chloride (VC), resulting in the production of ethene (a non-toxic product) as shown below. *Dhc*-like microorganisms are present at field sites where complete dechlorination has been observed, and are known to be present in all

commercially-available bioaugmentation cultures. Researchers continue to find new species of *Dehalococcoides* with varying treatment capacities.



Reductive Dechlorination Reaction Sequence for Chlorinated Ethenes

Bioaugmentation is an in situ remediation approach where complete dechlorination of chlorinated ethenes is stimulated by supplying microorganisms that have demonstrated the ability to completely dechlorinate chlorinated ethenes in the presence of the appropriate electron donors and nutrients. Bioaugmentation for remediation of chlorinated solvents in groundwater is a relatively new concept and a review of the current state of practice was provided by ESTCP (2005a). Bioaugmentation will continue to evolve from both the composition of cultures and an application perspective. Bioaugmentation cultures cannot partition into DNAPL phase liquid and as such rely on the dissolution of the compound into the aqueous phase.

1.2 Report Structure

This document provides the rationale for bioaugmenting sources (Section 2), technology evaluation, application and performance (Section 3), and references (Section 4).

2. RATIONALE FOR BIOAUGMENTING SOURCES

A number of field and laboratory studies have demonstrated that the activity of dechlorinating microorganisms is not inhibited at high chlorinated ethene concentrations that is typical of DNAPL sources, which indicates that these microorganisms can be active in close proximity to DNAPLs (Cope and Hughes, 2001; Carr et al., 2000; Yang and McCarty, 2002). Even if microorganisms cannot survive at the highest concentrations, researchers have shown that their activity will still result in enhanced dissolution rates of DNAPLs (Amos et al., 2007).

There are two principal technical reasons to consider bioaugmentation of DNAPL source zones:

1. Indigenous microorganisms cannot function at high enough concentrations to enhance dissolution rates of DNAPLs; and
2. Stimulation of indigenous microorganisms that can function at high concentrations will cause incomplete dechlorination. The enhanced dissolution rate of a single compound DNAPL will be substantially enhanced by the first dechlorination step (e.g., PCE to TCE and TCE to cis-1,2-DCE). However, if further dechlorination is not achieved there will be an increase in the mass flux of partially dechlorinated solvents that can cause plumes to expand. Complete dechlorination is necessary to contain the increase in mass flux.

Reasons to consider the application of bioaugmentation include:

- **Bioaugmentation is a feasible technology with low risk.** To date, bioaugmentation has been applied at over 100 sites in the United States where groundwater contains chlorinated ethenes. Many success stories with this technology have been documented in the peer-reviewed scientific literature and in conference proceedings (e.g. Major et al., 2002; Lendvay et al., 2003; Voci et al., 2004). A summary of the state of bioaugmentation, including current status and research needs, was prepared by ESTCP (ESTCP, 2005a and b).
- **Lack of appropriate dechlorinating microorganisms that function at high concentrations or where requisite *Dehalococcoides* organisms are absent or poorly distributed.** At these sites, bioaugmentation may be used to ensure that the necessary microorganisms to achieve complete dechlorination to ethene are present or to supplement the activity of the existing dechlorinating population.
- **Reduction of lag times to meet goals.** The presence of *Dehalococcoides* organisms at a site suggests that bioaugmentation may not be required for complete degradation

of chlorinated ethenes. Nevertheless, some sites where *Dehalococcoides* is present may benefit from bioaugmentation to decrease the lag time prior to the onset of dechlorination. For example, Lendvay et al. (2003) demonstrated that bioaugmentation decreased the time to achieve complete dechlorination to ethene from twelve weeks to six weeks; a benefit that may be significant when travel times to compliance points are insufficient, or where there are stringent regulatory or commercial deadlines.

- **Relatively low cost.** Bioaugmentation costs are often low relative to the cost of electron donor addition and it will improve dechlorination rates in the areas of interest.

3. TECHNOLOGY EVALUATION, APPLICATION, AND PERFORMANCE

This section presents how to assess the need for bioaugmentation (Section 3.1), factors affecting performance of bioaugmentation (Section 3.2), bioaugmentation application (Section 3.3.), performance of bioaugmentation of source treatment (Section 3.4), tools to track bioaugmentation performance (Section 3.5) and summary and conclusion (Section 3.6).

3.1 How to Assess the Need for Bioaugmentation

Currently, there are three ways to assess the need for bioaugmentation including: (i) when molecular analyses indicates low or no densities of *Dehalococcoides* microorganisms; (ii) when microcosm testing does not confirm presence of dehalorespiring microorganisms.; and (iii) interpretation of field geochemical data. These methods can be used independently; however, combining assessment methods will lead to a stronger conclusion. The basis, benefits and limitations of each assessment method are discussed in the following sections.

3.1.1 Direct Detection

Molecular analytical techniques are required to detect *Dehalococcoides* and other dechlorinating microorganisms because of their unique growth requirements; their syntrophic association with other anaerobic microorganisms precludes using conventional microbial detection techniques such as agar plate counts. A review of the various methods has been prepared by ESTCP (2005b). In general, the analysis involves the extraction and detection of unique gene fragments of *Dehalococcoides* or other halo-respiring microorganisms. Common molecular techniques use the polymerase chain reaction (PCR) to amplify the target gene fragment to detectable quantities. Quantitative (qPCR) PCR methods provide the number of gene copies per gram or liter of water. Generally, one gene copy is assumed to be equivalent to the number of microorganisms that contain that gene.

The direct detection of *Dehalococcoides* or other dechlorinating microorganisms indicates the potential to achieve complete dechlorination or partial dechlorination at a site, respectively. There are differences in the ability of different strains of *Dehalococcoides* organisms to halo-respire chlorinated ethenes. Some strains can convert cis-1,2-DCE to only VC, whereas others can completely convert cis-1,2-DCE and VC to ethene. Those strains contain the VC-reductase gene which can be assayed for by qPCR methods.

Negative detection of *Dehalococcoides* organisms may be a result of the detection limit of the assay or due to sampling bias. Detection limits are not as much of a concern because PCR assays can detect as few as 1000 gene copies per liter; however, due to sampling bias, a

particular sample might not contain *Dehalococcoides* DNA, even at sites that contain this organism at other locations. Therefore, the absence of detectable *Dehalococcoides* DNA over several site samples is suggestive (but not conclusive) that *Dehalococcoides* organisms are absent from the entire site.

3.1.2 Microcosm Testing

Prior to the development of molecular assays, microcosms containing site soil and groundwater were the standard approach for assessing the presence of dehalorespiring microorganisms. Assessing the need to bioaugment a given site involves comparing the rate, extent, and acclimation period (time to initiate reductive dechlorination and achieve complete dechlorination to ethene) of dechlorination between microcosm treatments. Electron-donor amended microcosms that do not proceed past cis-1,2-DCE after several (four to six) months of incubation indicate that bioaugmentation is required. Various published protocols and literature exist that describe standard procedures for microcosm studies (ITRC, 1998; AFCEE, 2004).

3.1.3 Biogeochemical Indicators

There are various geochemical conditions that indicate a need for bioaugmentation. These include:

1. Aerobic or anoxic environments with little or no evidence of anaerobic redox processes (i.e. nitrate, iron, manganese or sulfate reduction). Such conditions likely will not have provided the opportunity for Dhc to become established;
2. Sites where reductive dechlorination is occurring naturally with cis-1,2-DCE being produced but no VC or ethene are observed. Of note, VC and ethene can be formed naturally through either abiotic processes or anaerobic co-metabolic reactions. Accordingly, the presence of VC and ethene should not be used to infer that dechlorination will proceed to completion unless they constitute a significant fraction of the total chloroethene concentration (e.g., >10%); and
3. Production of VC or ethene is not observed within a period of time (e.g., 4 to 6 months) after establishing appropriate reducing conditions. Based on an assumed doubling time of 10 days, increasing the indigenous population of Dhc-like microorganisms from 100 cells/mL to 10^6 cells/mL is a sufficient biomass density to cause observable production of VC or ethene.

3.2 Factors Affecting Performance of Bioaugmentation

There are a number of factors that can affect the performance of a culture. These factors should be assessed in the remedial selection process. Conditions that may impact the proliferation and growth of *Dehalococcoides* organisms are shown in the following table.

Table 3-1: Impacts on *Dehalococcoides* with Various Groundwater Conditions

Groundwater Condition	Impact on <i>Dehalococcoides</i>
Low pH ¹ (typically less than 6 s.u.)	Dechlorination of VC to ethene may be slow at low pH. Bioaugmentation can be completed but VC may persist in groundwater. Note that dechlorination within source areas can reduce the pH over time, and buffering may be required.
Salinity	It is not clear if salt water intrusion affects degradation rates of amended cultures.
Dissolved Oxygen/Redox ²	Most <i>Dehalococcoides</i> organisms are sensitive to oxygen. Groundwater that has minimal dissolved oxygen and reducing conditions is preferable.
Temperature ³	<i>Dehalococcoides</i> organisms are mesophiles and cannot tolerate environments with extremely high (i.e., >45 °C) or low (i.e., < 5°C) temperatures.
Sulfate/Sulfide ⁴	In some instances, sulfate appeared to inhibit dechlorination. Recent work by Hoelen and Reinhard (2004) and Jeoun and Hayes (2003) indicate sulfide may be responsible for this inhibition.
Other VOCs	Some cultures are sensitive (i.e. have reduced dechlorination activity) when other VOCs are present. These can be 1,1,1-trichloroethane (1,1,1-TCA) or chloroform. As well, other VOCs in much higher concentrations (e.g. chlorinated methanes) may inhibit dechlorination activity in cultures. Check with the vendor as they may have specialist cultures to deal with mixed cVOCs.

1. Rowlands, 2004.
2. Maymo-Gatell et al., 1997; He et al. 2003; Seepersad, 2003.
3. Friis et al., 2007.
4. Hoelen and Reinhard, 2004; Jeong and Hayes, 2003.

3.3 Bioaugmentation Application

Figure 1 provides a general overview of the implementation stages of selecting a bioaugmentation approach for a source zone remedy. Each source zone will have its own site specific characteristics that will need to be assessed and this will guide the applicable source treatment technologies. Figure 1 assumes that the outcome of assessing treatment options is favorable for bioremediation as source treatment technology. Baseline conditions should be established as part of pre-design. Once a treatment area has been defined, an estimate of the mass of VOCs to be treated should be calculated. Based on the mass and the distribution in the treatment area, the metrics to assess the performance should be established. Examples of strategies to monitor DNAPL source remedies were summarized by ITRC (2005). Section 3.4 (below) provides more detail on assessing performance of bioaugmentation.

Most source areas lack sufficient electron donor to promote dechlorination and therefore the first step of the remedial design will be to determine the application methods and type of electron donor to the source zone. There are numerous reviews on the application of electron donors to subsurface settings (e.g., AFCEE, 2004).

The following section reviews the basis for selecting a dechlorinating culture. Appendix A contains a listing of the commercially available cultures and the stepwise application of one *Dhc* culture. After bioaugmentation is completed, the iterative process of performance assessment would begin.

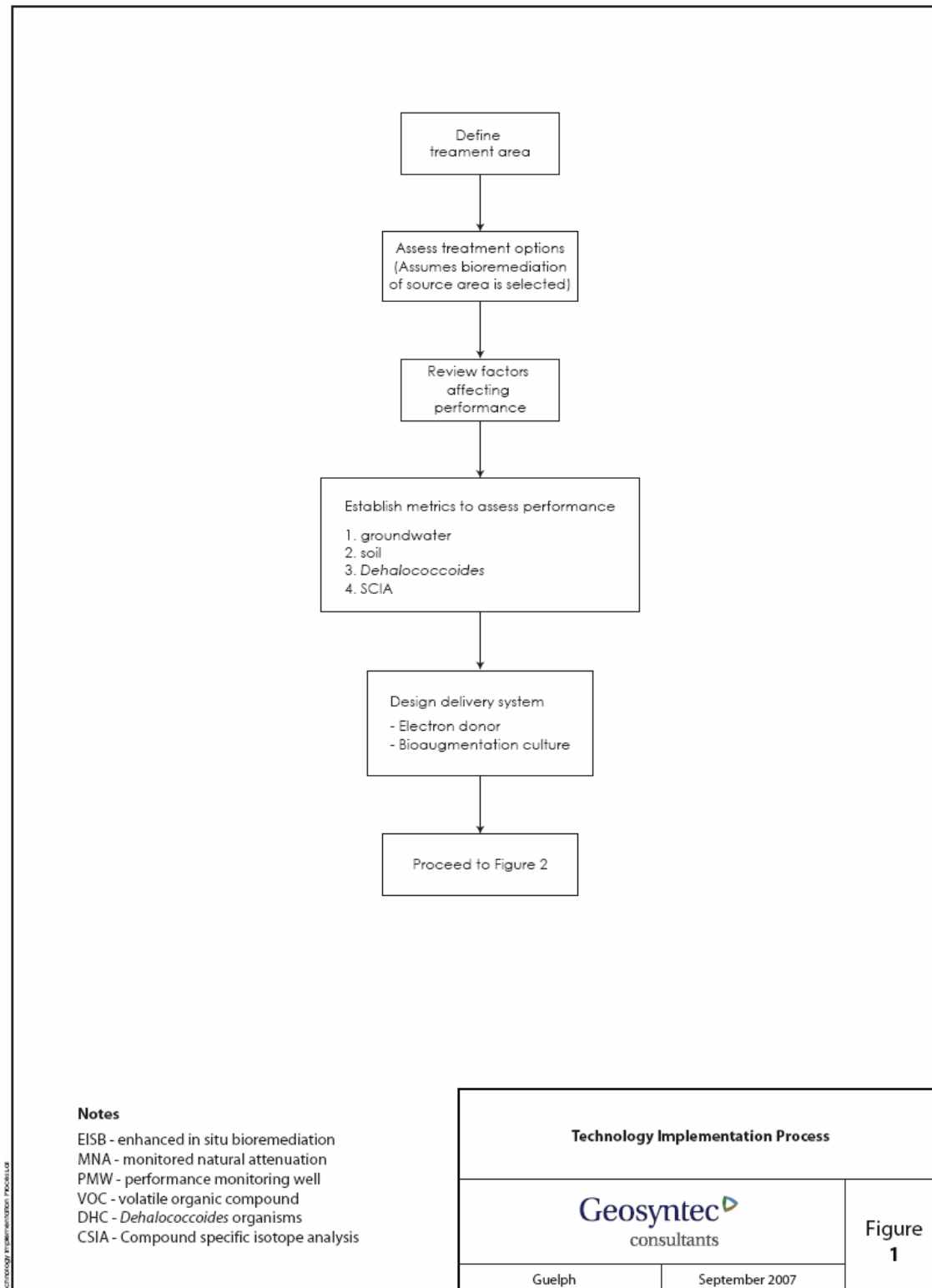


Figure 1

3.3.1 Selecting a Dechlorinating Culture

Baseline evaluation of the site would have identified the target VOC compounds requiring treatment. During design, one step will be to assess the ability of commercially available cultures to degrade the compounds of interest. Most commercially available cultures are mixed cultures (i.e. not a single pure species) and most contain *Dehalococcoides* organisms of slightly different types. There is currently no standard on what should define a bioaugmentation culture and each vendor is able to make claims as they see fit. Most vendors assist clients with determining the approximate volume needed to treat the area of interest. The following is a list of criteria that should be addressed by the vendor during the culture selection process:

- The approximate cell density of *Dehalococcoides* organism in shipped culture should be specified. Documentation should be provided that confirms the cell density in the materials provided and confirms that the shipment possesses dechlorination activity of the target VOCs;
- Cultures should be shipped without VOC compounds present so that these are not amended to the site. Documentation should be provided to confirm this;
- Cultures should be provided with assurances that they do not contain known pathogens. Documentation should be provided to confirm this; and
- Shipping, storage and handling procedures for the equipment supplied by the vendor should be provided.

Once a culture has been selected, the conditions of application should be confirmed (see Section 3.2) with the vendor. Appendix B contains the Bioaugmentation Check List that is provided by one vendor (SiREM). This approach was used in the successful bioaugmentation of a PCE source as outlined in the ER-0008 final report (NFESC and Geosyntec, 2007).

3.4 Performance of Bioaugmentation of Source Treatment

There are no standard protocols for measuring the performance of DNAPL source zone treatment technologies but there are a variety of assessment tools, including groundwater sampling, soil collection, enhancement factors and, stable compound isotope ratios that can provide information about the changes in concentration of contaminants in groundwater or the amount of mass remaining in the source zone.

Assessing the performance of enhanced in situ bioremediation (EISB) effectiveness in source zone remediation can be very different from other DNAPL remediation strategies from an implementation perspective. Many DNAPL technologies are aggressive and are applied as one-time, short duration actions that last from weeks to months; whereas EISB as a source

remedy will be applied over months to several years. Therefore, it is important to not monitor in excess, but to collect metrics that are meaningful on a time frame that is appropriate for the site specific source.

The goal of bioaugmentation in source treatment should be to enhance mass transfer and ultimately accelerate the dissolution of the residual DNAPL. There are various metrics that are useful to assess performance of bioaugmentation and source treatment, including:

- Changes in VOC concentrations and mass flux that are consistent with biological processes within and downgradient of the source area;
- Molecular tools (see Section 3.5.2); and
- Stable isotope ratios (see Section 3.5.3).

Figure 2 provides an example of the performance assessment process for a source area bioaugmentation. In this process, the Remedial Program Manager (RPM) needs to assess the system performance. The following sections review key indicators of performance.

- **System performance.** There needs to be an on-going evaluation of the residual electron donor capacity. Routine measurements should be taken to ensure the system is not electron donor limited. Things to evaluate would include assessing the proximity of donor to source and the proximity of *Dhc* to source. All of these factors can impact performance. As well, the factors that would affect performance (Section 3.2) should be evaluated routinely after system start up.
- **Enhancement factor.** As shown in Figure 2, once there are increases in degradation products (e.g., cVOCs) or *Dhc* is observed, then the VOC mass flux changes or concentration changes can be monitored. Flux meters, fences or tracer tests can all help capture flux changes. Determining the amount of enhancement from bioaugmentation will facilitate the comparison of the equivalent VOC concentration to that from baseline. If there is an increase in mass transfer, then the consistency of this enhancement can be monitored if feasible.
- **Complete dechlorination.** The extent of dechlorination is also important. Monitoring should include sampling for ethene or other terminal end products (e.g., chloride). If electron donor or *Dhc* distribution is not optimal, there may only be the presence of intermediate degradation products. Intermediate degradation products are beneficial as they can change the concentration gradient and encourage DNAPL dissolution; however, sufficient donor then needs to be present down gradient to allow for complete dechlorination (e.g., passive permeable barriers could be installed some distance from the source to provide secondary treatment of degradation products).

- **Distribution and activity of dechlorination.** Understanding the distribution of the dechlorinating organisms in the source area can help understand why dechlorination is or is not occurring to the extent predicted. Molecular biological tools (MBTs) (see Section 3.5.2) are useful tools for assessing the level of activity and the distribution.
- **Tracking source depletion.** A key metric is being able to gauge when a source may be depleted. Residual pools are difficult to locate and often the reason why defining a source area can be very costly. Morrill et al. (2003) demonstrated that the parent stable carbon isotope analysis from aqueous groundwater samples remains unchanged while DNAPL is present (as there is a constant pool of parent compound dissolving). Using the ratio of ^{12}C to ^{13}C at a subset of monitoring wells, proximal to the source area, will help to track source depletion.

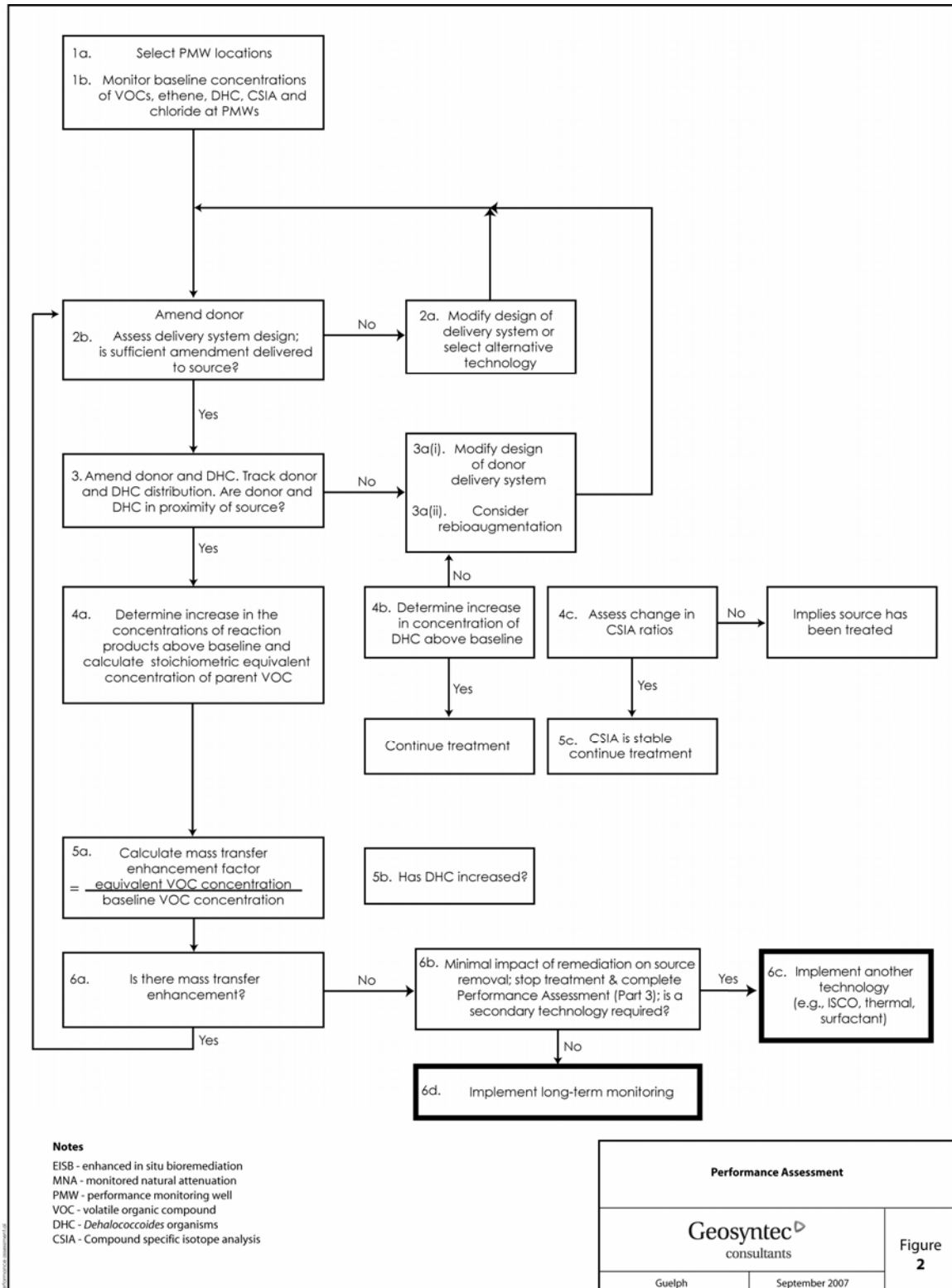


Figure 2

3.5 Tools to Track Bioaugmentation Performance

The following sections provide a summary of culture quality assurance/quality control, MBTs and stable compound isotopes analyses.

3.5.1 Quality Assurance/Quality Control (QA/QC) for Cultures

The potential for adverse impacts to groundwater quality during bioaugmentation caused by the inadvertent introduction of pathogenic or opportunistic microorganisms represents a significant potential concern. Equally important is the potential for contamination or other changes to the microbial community composition that may adversely impact the dechlorinating activity of a bioaugmentation culture. The implementation of QA/QC protocols to prevent these impacts will encourage the effective application of bioaugmentation and, in the longer term, increase the acceptance of bioaugmentation as a credible remediation technology.

At a minimum, protocols for the production and shipment of bioaugmentation cultures to field sites for application should ensure that:

- The degradative activity and composition of each batch of the culture are comparable with that of the original culture for which reliable field performance and characterization data are available;
- The degradative microorganisms in the culture are not displaced or reduced in number by pathogenic or other opportunistic microorganisms during production, shipment, or introduction to the subject site;
- The composition and density of the culture is uniform between successive production batches; and
- The viability and activity of the culture at the time of injection are at the highest possible levels.

3.5.2 Molecular Biological Tools

Molecular Biological Tools (MBTs) target process-specific biomarkers or specific organism(s) that complete or mediate a process of interest (e.g. dehalogenation of VC to ethene). Most MBTs target nucleic acids (i.e. DNA and RNA); however, lipids and proteins could be targeted as well. The most widely used biomarker to qualitatively assess the presence of a specific bacterial group is the 16S rRNA gene (Pace et al. 1986; Stahl 1997). A variety of methods, including PCR, terminal restriction fragment length polymorphism (T-RFLP), denaturing gradient gel electrophoresis (DGGE), and fluorescent in situ hybridization (FISH), have become routine procedures to assess a sample for the presence of a particular 16S rRNA

gene belonging to an organism or a group of organisms of interest. Appendix C contains a summary of the techniques and commercial laboratories which offer MBT-related services.

MBT analyses may be relatively expensive compared to other types of analyses (e.g. VOCs), although the information that they provide is often well worth the added cost. Thus, it is important that the client consider the following steps in collecting MBT samples for analysis: (i) the MBT(s) most suited to the question of interest (e.g. PCR, FISH, DGGE); (ii) sampling location(s); (iii) an appropriate sampling procedure; and (iv) an appropriate sample handling and storage procedure. It is important that these steps are completed so that the biomarkers remain intact before reaching the analytical laboratory. Protocols and procedures which have industry "acceptance" do not yet exist. Strategic Environmental Research and Development Program's (SERDP) project ER-1561 will assess standardization of MBTs. Commercial MBT laboratories often will provide support or guidance on recommended sampling methods for the various MBTs. SERDP has also developed an excellent document reviewing the current use and applicability of MBTs (ESTCP 2005b).

It is recommended that RPMs have a clear objective in using MBTs and review both vendor materials and peer-reviewed publications to determine if the MBT of interest is appropriate to answer the questions being posed. As with other analytical techniques, it is important to conduct a baseline MBT analysis prior to remedial activities to which later MBT results can be compared. Thus, MBT analyses should be considered early in the site remediation process.

3.5.3 Stable Compound Isotope Ratios

Isotopes of carbon, hydrogen, nitrogen and other compounds can be used as an indicator of biodegradation reactions. Slater (2003) provides a summary of the process, case studies and applicability of stable compound isotopes for bioremediation. Chlorinated solvent molecules are composed of light and heavy atoms; for carbon, this is ^{12}C and ^{13}C carbon atoms. During biodegradation, the ratio of heavy to light isotopes changes, as biological processes will preferentially transform lighter compounds over heavy ones. This results in a progressive enrichment of the heavy isotopes in the remaining contaminant pool, with respect to the initial isotopic signature. In DNAPL settings, the isotopic signature will remain constant, as there is a continuing supply of cVOCs. Once the DNAPL has been dissolved, the parent cVOCs isotopic signature becomes enriched (Morrill et al., 2003; ETSCP Final Report on ER-0008). Stable compound isotopes can be a useful tool to track source depletion when using bioremediation and should be included in the overall performance assessment strategy.

At baseline, a groundwater sample can be collected from several performance monitoring wells, and be assessed for the initial carbon isotope signature. This initial isotopic signature should remain constant until the source is depleted. However, this tool cannot predict when dissolution will be completed but it can indicate the rate of change if sampled at the same

location over time. Commercially, one analytical laboratory offers these services, in addition to several research laboratories (see Appendix C).

3.6 Summary and Conclusions

Several studies, including this ESTCP project (ER-0008) have proven that bioaugmentation of source areas is technically feasible. The enhanced dissolution rate of a single compound DNAPL will be substantially enhanced by the first dechlorination step (e.g., PCE to TCE and TCE to cis-1,2-DCE). However, if further dechlorination is not achieved, there will be an increase in the mass flux of partially dechlorinated solvents that can cause plumes to expand. Complete dechlorination is necessary to contain the increase in mass flux.

Bioaugmentation is a feasible technology with low risk. To date, bioaugmentation has been applied at over 100 sites in the United States where groundwater contains chlorinated ethenes. Factors to consider for the application of bioaugmentation include:

- **Lack of appropriate dechlorinating microorganisms that function at high concentrations or where requisite *Dehalococcoides* organisms are absent or poorly distributed.** At these sites, bioaugmentation may be used to ensure that the necessary microorganisms to achieve complete dechlorination to ethene are present or to supplement the activity of the existing dechlorinating population.
- **Reduction of lag times to meet goals.** The presence of *Dehalococcoides* organisms at a site suggests that bioaugmentation may not be required for complete degradation of chlorinated ethenes. Nevertheless, some sites where *Dehalococcoides* is present may benefit from bioaugmentation to decrease the lag time prior to the onset of dechlorination. A benefit that may be significant is when travel times to compliance points are insufficient, an increase in mass flux will cause expansion of a plume of partially dechlorinated products, or where there are stringent regulatory or commercial deadlines. Some sites may have long treatment times (e.g., 30 years) and in these cases, the benefit of bioaugmentation will need to be considered over the lifetime of the project.
- **Relatively low cost.** Bioaugmentation costs are often low relative to the life cycle costs of the remedy (including capital costs, electron donors and their addition, and routine operation and monitoring costs) and it will improve dechlorination rates in the areas of interest.

Issues to be considered in the application of a bioaugmentation culture to a source zone include:

- Factors impacting *Dehalococcoides* with Various Groundwater Conditions (refer to Table 3-1);
- Designing the electron donor and bioaugmentation application methodology (passive versus induced gradient/recirculation);
- Tracking bioaugmentation performance (Section 3.4); and
- Tools to track bioaugmentation performance (Section 3.5).

The assessment of the technology is reviewed in Figure 1 and an example of implementation strategy is provided in Figure 2.

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APPENDIX A

List of Commercially Available Bioaugmentation Cultures for

Chlorinated Ethene Degradation

APPENDIX A: LIST OF COMMERCIAL AVAILABLE BIOAUGMENTATION CULTURES FOR CHLORINATED ETHENE DEGRADATION
ESTCP Bioaugmentation of DNAPL Source Zone Areas: Lessons Learned on ESTCP ER-0008

Name of Company	Address	Phone Number	Web Page	Bioaugmentation Cultures Available
Bioremediation Consulting Incorporated	39 Clarendon St. Watertown, MA 02472	(617) 923-0976	http://www.bcilabs.com/s.bioaug.html	<ul style="list-style-type: none"> ● BCl-e - <i>Dehalococcoides ethenogenes</i> (in mixed culture) ● BCl-a - <i>Dehalococcoides ethenogenes</i> (in mixed culture) ● BCl-t - <i>Dehalococcoides ethenogenes</i> (in mixed culture)
Terra Systems, Inc.	1035 Philadelphia Pike, Suite E Wilmington, DE 19809	(302) 798-9553	http://www.terrasystems.net/	<ul style="list-style-type: none"> ● Pinellas culture (licensed from GE)
Shaw Environmental Inc.	17 Princess Road Lawrenceville, NJ 08648	(609) 895-5350	http://www.shawgrp.com/markets/envservices/envtechnology/techbioaug	<ul style="list-style-type: none"> ● SHAW SDC-9 Culture
Regenesis	1011 Calle Sombra San Clemente, CA 92673	(949) 366-8000	http://www.regenesis.com/products/bioAug/	<ul style="list-style-type: none"> ● Bio-Dechlor INOCULUM® ● Bio-Dechlor INOCULUM® PLUS(+)
Bioremediation and Treatability Center (BTC)	25 Spring Street Walpole, MA 02081	(508) 668-0191	http://www.biotreatcenter.com/services.htm	<ul style="list-style-type: none"> ● NJ-14 Mixed Culture ● BW-05 MTBE Culture ● SL-D Culture
SiREM	130 Research Lane, Suite 2 Guelph, Ontario, Canada N1G 5G3	(519) 822-2265	www.siremlab.com	<ul style="list-style-type: none"> ● KB-1® ● KB-1® Plus ● WBC-2 (under CRADA with USGS)

APPENDIX B

Bioaugmentation Check List (Sample Checklist from SiREM)

MEMORANDUM

TO: Michayae McMaster, Geosyntec Consultants
FROM: Sandra Dworatzek, SiREM
DATE: 04 July 2007
SUBJECT: Injection Procedure for KB-1®

List of Equipment

- Pressurized cylinder of compressed gas (argon or nitrogen) – to be provided by customer.

KB-1® injection field kit provided by SiREM containing equipment and materials required for injection:

- Stainless steel vessel containing KB-1® Dechlorinator
- Appropriate regulator for compressed inert gas
- Tubing with appropriate fittings for transfer of gas to vessel and inoculum from vessel to well.
- Tubing to inject inoculum into subsurface (use dedicated tubing for each well).
- Digital scale (to measure KB-1® injection volumes)

Summary of Method

SiREM ships all required equipment and materials to the site prior to the scheduled injection date. The bioaugmentation technician inspects the KB-1® vessel integrity upon arrival at the field site and confirms that all valves are closed and all connections are secure. The pressure in the KB-1® vessel and vessel weight are checked to ensure that they have not changed since leaving the laboratory. The vessel is shipped under slight positive pressure (1 pound per square inch [psi]).

The injection technician proceeds with the injection by placing the injection tubing in the well to the desired injection depth and purges the well with argon or nitrogen gas to displace oxygen from the well column and maintain an inert gas blanket in the well above the water table. A 5 minute purge is recommended.

KB-1® is then injected into well/drive point using compressed gas. There are four stainless steel stems on SiREM KB-1® vessels: the pressure relief and pressure gauge line (to monitor pressure and pressure relief for safety); the perforated line (not for use in field, lab use only); the inoculation line (culture line into subsurface); and the vent line (connected to compressed gas to pressurize vessel and push culture into the ground) shown in Figure 1. The bioaugmentation technician connects the tubing so that the compressed gas is used to pressurize the KB-1® vessel (typical maximum pressure required is up to 30 psi for injection depths up to 30 feet below ground surface) and push the KB-1 culture into the injection tubing and into the well/drive point at the desired depth

interval. The required volume is metered into the ground and the injection equipment moved to the next injection as required until all locations are completed.

The bioaugmentation technician facilitates the return of all materials and equipment to SiREM following completion of the bioaugmentation.

Health and Safety Considerations:

Appropriate protective eyeglasses or goggles are to be worn when opening KB-1 vessels valves or pressurizing vessels when injecting contents in to groundwater. Disposable latex or nitrile gloves should be worn when handling and disposed of after use.

Spilled liquid should be soaked up with a sorbant and saturated with a 10% bleach solution (1/10 diluted standard bleach). Sorbant should be double bagged and disposed of in garbage. After removal of sorbant, area should be washed with 10% bleach solution to disinfect. If liquid from the culture vessel is present on the fittings, non-designated tubing or exterior of the culture vessel liquid should be wiped off and the area washed with 10% bleach solution.

KB-1® Vessel
 Empty Weight = 27 lbs
 Full Weight = 77 lbs



33"

28"

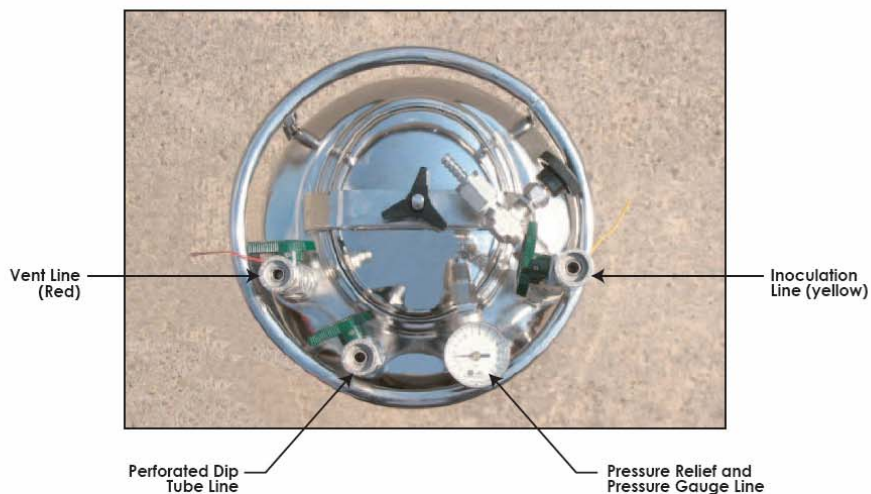
9.3"

KB-1® Vessel Shipping Case
 Empty Weight = 28 lbs
 Full Weight with KB-1 Vessel = 105 lbs



36"

18" 16"



KB-1® Vessel and Shipping Case

Feb. 2007

Figure: 1



APPENDIX C

List of Commercial Laboratories Offering Molecular Biological Tool Analytical Services

APPENDIX C: LIST OF COMMERCIAL LABORATORIES OFFERING MOLECULAR BIOLOGICAL TOOL ANALYTICAL SERVICES
ESTCP Bioaugmentation of DNAPL Source Zone Areas: Lessons Learned on ESTCP ER-0008

Name of Lab	Address	Phone Number	Web Page	Services Provided
Microbial Insights, Inc.	2340 Stock Creek Blvd. Rockford, TN 37853-3044	(865) 573-8188	www.microbe.com	<ul style="list-style-type: none"> • Quantitative PCR(Q-Potential/Q-Expression) • Denaturing Gradient Gel Electrophoresis • Phospholipid Fatty Acid (PLFA) • Stable Isotope Probing • Protein Mass • Isolate ID
Bioremediation Consulting Incorporated	39 Clarendon St. Watertown, MA 02472	(617) 923-0976	www.bcilabs.com/s.pcr.html	<ul style="list-style-type: none"> • PCR based testing for Dehaolococcoides and Dehalobacter
GAP Enviromicrobial Services	1020 Hargrieve Road, Unit 14 London, Ontario, Canada N6E 1P5	(519) 681-0571	www.gapenviromic.com/biodegradation.htm	<ul style="list-style-type: none"> • PCR based testing for Dehaolococcoides
SiREM	130 Research Lane, Suite 2 Guelph, Ontario, Canada N1G 5G3	(519) 822-2265	www.siremlab.com	<ul style="list-style-type: none"> • qPCR based testing for Dehaolococcoides, vinyl chloride reductase and Dehalobacter • Denaturing Gradient Gel Electrophoresis